Development of Bait Stations for Fruit Fly Population Suppression

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ABSTRACT The application of insecticides is an essential component for eradication or management of fruit fly pests. Impact on nontarget organisms and public rejection of areawide pesticide applications have been major concerns in managing these programs. Bait stations have been proposed as alternative treatments in areas where broadcast insecticides are not acceptable. In this study, we defined bait stations as discrete containers of attractants and toxins, which are targeted at specific pests. Tests were carried out using the Mexican fruit fly, *Anastrepha ludens* (Loew), as the experimental insect. Our first bait station design was a sheet of sponge material fastened to a plastic peaked cover. Liquid bait consisting of protein hydrolyzate, sugar, adjuvants, a photoactive dye toxicant, and other additives was applied to the sponge. This station, when tested in an orchard, reduced sterile released adult populations by 70–90% in 4 d compared with check plots. Other tests in field cages showed that the bait station was ≈22% less effective in killing adults compared with spot sprays on trees. We formulated a gelled bait by using a more refined hydrolyzed protein, supplemental attractants, feeding stimulants, and additives to protect the bait from drying. A series of experiments were carried out in field cages by using a cylindrical bait station that provided improved protection of the bait. These tests showed that there is a gradual decline in bait effectiveness with age.

KEY WORDS *Anastrepha ludens*, Mexican fruit fly, GF-120, Solbait

Insecticide bait sprays have been major control methods for managing or eradicating tropical fruit flies (Back and Pemberton 1918, Crawford 1927). Public concerns about ecological and health impacts from application of these have been an issue for state and federal agencies responsible for fruit fly control in the United States (EPA 2005). In the United States, most states involved in eradicating outbreaks of tropical exotic fruit flies have protocols using a combination of ground-applied spot sprays in sensitive zones, such as residential areas, near water bodies, or areas around hospitals, schools, and parks; and aerial coverage for large agricultural areas where complete coverage is acceptable. Use of spot spray application does not negate the problem of bait and insecticide release into the environment and possible undesirable impact on property, wildlife, and human reactions to the chemicals.

Protein-based baits presented in field sites in small discrete containers are widely used in fruit fly management programs, but experimental evidence verifying their impact is lacking. According to weekly reports from the Guatemala–Mexico–United States Mediterranean fruit fly (Mosca Med) program, these stations were used over thousands of hectares of host material for eradication of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 1983, Orozco et al. 1994, Tween 2004). During the 2002 outbreaks of Mediterranean fruit fly in the Palenque region of Chiapas, Mexico, 164,241 bait stations were deployed over 8,332 ha. In a larger treated area, the Guatemala program deployed 36,183 bait stations. Most of these stations were composed of absorbent material such as plastic sponge, corncob, or banana leaf fiber, soaked in a liquid mixture of malathion and an acid hydrolyzed protein bait (Captor 300). Some stations were protected from rain by a plastic disc or inverted cup attached to a hook attaching the station to the host plant.

Methods of application or quality control in use of bait stations are not presented in official work plans or documentation of these programs. The technique was described in documentation provided in an international course dealing with fruit flies by the Mexican Department of Agriculture (SAGAR) (Arjona 2000). That report described bags filled with locally available absorbent materials (ground corncobs or cotton material) soaked in the same bait used in the sprays. We also have observed use of corncobs or banana leaf strips soaked in the bait. These stations were hung or thrown in trees at 30-m intervals or at an approximate density of nine stations per hectare. The persistence of bait activity was not described in this report.

The bait stations currently used in Guatemala for Mediterranean fruit fly control and in programs in northeastern Mexico for *Anastrepha* spp. (R.L..M., personal observation) use the same attractant–insec-

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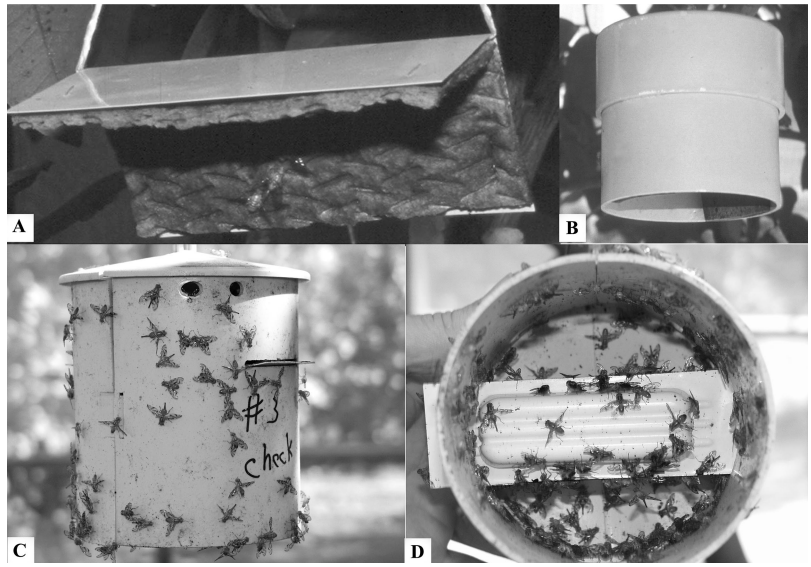


Fig. 1. Photographs of three bait station models tested. (A) Mazoferm station consisting of sponge sheet fastened to plastic sheet. (B) PVC cylindrical station. (C) Commercial cylindrical station exterior with no insecticide check bait. (D) Commercial cylindrical station showing position of bait tray insert.

ticide combination that is used in bait sprays. In experiments presented here, we tested liquid formulations in bait stations designed to perform in a manner similar to stations currently used in Mexico and Guatemala to evaluate Mexican fruit fly, *Anastrepha ludens* (Loew), mortality. These tests were followed by evaluations of the liquid formulation augmented with additional attractants, conditioners, and thickeners to enhance attraction and extend persistence of the baits.

Materials and Methods

Our efforts to develop bait stations began with tests of Mexican fruit fly attraction and feeding on hydrolyzed protein baits in field cages. We used bait stations to minimize contaminating the cage and trees with attractants or insecticides. Later trials compared mortality and survival of released Mexican fruit flies to sprayed nursery trees or bait stations hung in the trees in adjacent cages.

The field cages were constructed in the USDA–ARS Rio Red grapefruit orchard in Weslaco, Hidalgo County, TX. All orchards were flood irrigated according to standard practices in the region. No insecticide treatments were applied for fruit fly control. Occasional treatments for mite and scale insect control were made but normally no insecticide treatments were made during the tests except where noted. Weather during these tests ranged from cool winter temperatures of <15°C at night up to 25°C during the day, and summer temperatures of 23°C at night to 40°C during the day. We attempted to carry out most experiments within the range of 18–32°C, because temperatures outside these extremes reduced fly activity.

The field cage tests were performed in two blocks of cages, each containing four individually caged Rio Red grapefruit trees. For each block, the cage was 3.73 m in height, two cages on the south side measured 6.51 by 4.76 m. The two cages on the north side measured 4.81 by 4.81 m. Trees in the cages were pruned to provide a solid canopy, but interior limbs and low hanging limbs below 1.2 m had been removed to provide access and visibility. Limbs also were pruned so that no foliage was in contact with the sides or top of the cage. In all cage tests, a water source was provided by hanging two 1-liter containers filled with water in each cage. Dental wicks extended from below the water surface to ≈8 cm from the tops of the containers and remained wet for the testing period.

Mexican fruit flies used in the orchard experiments were sterilized flies from a mass rearing strain maintained at Mission, TX, by USDA–APHIS–PPQ for the sterile insect release program in the Rio Grande Valley, and the California–Mexico programs. Cage release flies also were sterilized but were from more recently colonized (four to eight generations) strains collected in Nuevo Leon and Tamaulipas, Mexico.

Bait Station Designs. Three model stations were tested. The first model (Fig. 1A), referred to as a tent station, was a sheet of sponge attached under a folded sheet of plastic measuring 21.5 by 24.0 cm folded in half to make 90° angle. The plastic acted as a peaked protector with sloped walls each measuring 24.0 cm in width and sloping sides 10.7 cm in length. The sheet of sponge material (19 by 20 cm and 5 mm in thickness when dry, 8 mm in thickness when wet) was stapled to the underside of the tent to line the under surface leaving a 2.0-cm overhang of the plastic on all sides. These stations were placed in trees by using a hanger of wire or nylon cord placed between the sponge and the plastic sheet with ends attached to the limb of a tree. Stations were hung in trees ≈0.3 m inside the

Table 1. Components of baits used in bait station tests

Liquid Mazoferm bait		Gelled Solulys bait (Solgel)		Function
Ingredient	% vol or wt	Ingredient	% vol or wt	
Phloxine B	0.5	Phloxine B	0.5	Toxicant
Mazoferm	70.0	Solulys hydrolyzed protein	4.4	Attractant protein
Invertose sugar	20.0	Sucrose	30.0	Feeding stimulant
		Invertose sugar	20.0	Feeding stimulant
Polysorbate 60	1.0	Polysorbate 60	1.0	Adjuvant
Soybean oil	1.0	Peanut Oil	1.0	Antifoamant, conditioner
Acetic acid	0.6	Ammonium acetate	1.0	Attractant
Polyethylene glycol	2.0	Polyethylene glycol	1.0	Humectant
Xanthan gum	0.4	Vegetable gums and starches	26.7	Thickener, conditioner
Water	4.5	Water	14.4	Liquifier

canopy drip line and at ≈1.8 m high in the canopy. These stations were completely open at the bottom, and flies could enter and leave without barriers or traps. Bait was applied with a hand pump sprayer calibrated to apply 100 ml of liquid bait to the sponge. All tent station trials used the "Mazoferm" bait described in Table 1.

The second bait station model, the cylindrical station (Fig. 1B), was designed as a more enclosed structure that would better protect the bait. This station consisted of a polyvinyl chloride (PVC) pipe 10.1 cm i.d. and 10 cm in length with a 4.5-cm-deep cap covering one end. A ring bolt through the center of the cap provided a 1.5-cm-diameter ring to attach to a hanger. The inside surface of the pipe was fitted with a plastic support for a bait trough to extend across the i.d. of the pipe so the bottom of the trough was 2.5 cm above the lower lip of the cylinder. The trough was cut from 3.5-cm-o.d. PVC pipe split longitudinally and 9.5 cm in length to fit snuggly inside the station frame. Sheets of plastic were cut to cover the ends of the trough. Additional attractant packets could be attached to the inside walls of the station above the bait trough. All baits used in the cylindrical station were the Solulys-based gelled material described in Table 1. After pouring the bait into the trough, a 15-mesh nylon screen (9.5 by 3.5 cm) was set into the soft surface of the gel to provide a walking surface for the flies.

The cylindrical bait station was modified for commercial development as a third model. This configuration provided a housing (Fig. 1C) and supported trays (Fig. 1D) that could be filled with bait for efficient storage and transport. The station housing was designed as two hinged halves that opened longitudinally and snapped together. The bait tray was designed to slide into the cylinder with tabs that protrude through slots on the cylinder sides. The size and shape of the commercial version of this station was identical to the PVC version except that the top was a peaked conical shape. These stations were made of pale green plastic to minimize damage by vandals or theft.

Methods of Data Analysis. These experiments relied on designs that measured effects of treatments on mortality (dead flies counted) or survival (flies surviving treatments and retrapped) for known numbers of insects released into a field cage or isolated orchard. Analyses compared numbers of flies recorded in treatments compared with numbers in control cages or blocks for experiments replicated over time. We relied on simple analysis of variance (ANOVA) models to compare mean responses of treated and control tests for repetitions at different dates when possible. We also followed recommendations such as those given in Wilkerson et al. (1996) to avoid the use of mean separations statistics such as Duncan means tests. In some cases, such as the comparisons of tree sprays and bait stations, more complex designs were required because of limited numbers of cages. These methods are described below. Results were analyzed by ANOVA models or pairwise *t*-tests by using Systat 10.2 (Systat Software, Inc., Richmond, CA).

Mazoferm Bait Used in Tent Stations. The desirable traits of bait formulations include attraction, feeding stimulation, and persistence in tropical fruit fly environments. Earlier feeding studies (Moreno and Mangan 1995) had shown that the conventional acid hydrolyzed protein baits that had been developed for use with organophosphate, carbamate, or chlorinated hydrocarbon insecticides were not suitable for use with the safer pesticides that must be ingested for effect. The first bait (Table 1) we tested was composed of Mazoferm E802 (Corn Products, Argo, IL.) (Moreno and Mangan 2002), a corn condensate hydrolyzed by a *Lactobacillus* sp. that consists of amino acids, carbohydrates, vitamins, and minerals. This material was combined with phloxine B (the toxin), water, and a series of additives including antifoamants, thickeners, humectants, and organic acids.

Bait Stations Compared with Spot Sprays on Trees. Toxic baits are often applied to host trees as "spots" (e.g., Burns et al. 2001) in areas where broadcast applications of these materials are not desired or permitted. The spots normally range from 50 to 100 ml of bait and are applied via backpack or vehicle-mounted sprayer to an area of ≈2 m^2 on the foliage of the host plant or other surface. To mimic this system without contaminating our field cage trees, we placed potted nursery grapefruit trees ≈1 m in height (so that pot + tree were ≈1.2 m in height) on four sides of the caged trees. The nursery grapefruit trees were placed so that their top leaves were just below the lowest skirt leaves of the mature trees in the cage. Each nursery tree was sprayed with 50 ml of bait to mimic a spot spray system used in ground applications in residential areas in eradication programs. Bait station cages had two bait stations in each of the mature trees; each station had

100 ml of Mazoferm bait applied to the sponge. No nursery trees were placed in the bait station cages. Although the spot spray cages contained four baited trees and the bait station cages contained only two bait stations, the bait stations were located inside the mature tree canopy, which is the preferred roosting location of the flies.

In the two treatment blocks there were eight cages. Treatments were applied to have three cages of the toxin-treated trees or stations and a single cage with check (bait with no insecticide) trees and stations. Therefore, one four-cage block had one cage with trees sprayed with bait without insecticide, two cages with trees sprayed with toxic bait, and one cage with bait stations with toxin. The other block had one cage with stations containing bait only, two cages with bait stations with toxic bait, and one cage with trees sprayed with toxic bait. Flies were released on Monday at ≈0730 hours. Trees and stations were prepared outside the cage and placed in the cage immediately after treatment with the baits. Dead flies were collected from the floor of the cage at 2-h intervals until 1530 hours everyday until Friday afternoon. These flies were recorded as killed flies. Then, nursery trees, bait stations, and water sources were removed from all cages, and two McPhail traps with torula yeast bait were placed in each cage. Flies were trapped from 1530 hours Friday until 0700 hours Monday and recorded as survivors. Tests were performed in March, April, and May. Treatments were rotated between cage blocks and within the cage blocks so that data were recorded from both blocks of cages for each treatment.

We first calculated all main effects and interactions among treatment dates and types of insecticide treatments on counts of dead flies recovered and surviving flies trapped after treatment. The flies used on each date came from the same rearing group and we decided that, although the individual cages were independent observations; the fly populations in the separate cages were from the same rearing lot and were not from independent populations. Our ANOVA model used the counts of flies as the dependent term and the mean square for treatment and date as the independent variables with the treatment × date interaction as the error term. Separate ANOVAs were calculated for mortality (dead fly counts) and survival (flies recaptured after treatment). We also compared each toxic application with a control (complete bait without phloxine B) to determine the effects of the toxin in each treatment. Analyses were performed to compare effects of check (bait, no toxin) and toxic bait (bait with toxin) and effects of method of treatment (tree or station).

Field Trials with Mazoferm Tent Stations. Field trials were performed using the Mazoferm tent (Fig. 1A) bait stations in the grapefruit orchard adjacent to the field cage. This experiment involved dividing the orchard into two roughly equal sized (≈0.5 ha each) blocks of citrus, one block was treated with stations treated with bait and toxin, the other with check stations with only bait. Sixteen stations were hung in trees at ≈15-m intervals in four alternate rows of trees (four stations per row) in the center portion of each 12-row section. Thirty thousand sterile Mexican fruit flies were released in the total (≈1-ha) orchard. After 5 d, stations were removed, and 12 McPhail traps with standard torula baits were placed in the orchard and surviving flies were recaptured. These experiments were repeated for five treatments in October–November and three treatments the following August. Treatment positions in the plots were reversed on consecutive weeks. This procedure slightly biased the data because untrapped surviving flies in both insecticide and control treatments could survive into the following week. The bias was against the insecticide treatment because much larger numbers of flies were surviving in the control treatments from the previous week. Treatment effects were analyzed using pairwise *t*-tests to compare effects of toxic stations versus check stations on recapture rate.

Transferred Kill Mortality. We tested the possible effect of flies spreading the toxin and killing individuals that did not visit bait stations but ingested regurgitated droplets from flies that had fed at the stations. In these tests, populations were exposed to baits with insecticides or check baits without insecticide, after 2 d baits were removed and a second fly population was introduced. During a 1-wk period, two groups of flies were released into the cage. The first group of 1,500 flies marked with a red external dye, was released on a Monday and the standard Mazoferm tent stations were hung in the cages at the same time. Two field cages were check cages provided with stations containing bait but no insecticide. The other two cages were insecticide cages with each cage provided with two stations, the same bait, plus the insecticide phloxine B. After 2 d, the stations were removed and a second group of 1,500 flies with green marking was introduced. Two McPhail traps were placed in each of the cages late Friday afternoon and removed on the following Monday morning. Efficacy of flies spreading the dye to the second released group (green flies) was estimated by comparing survival rate of this second group between the insecticide and check cages. We replicated this experiment four times with treatments rotating among cages.

Addition of Synthetic Baits. Tests were performed to determine whether reduction in mortality rates in the Mazoferm bait stations over time was due to loss of attraction and feeding of the flies on the bait or due to loss of toxicity of the bait. A test series was designed to use synthetic bait, BioLure (Suterra LLC, Bend, OR) (Epsky et al. 1995, Heath et al. 1995) that was commercially available and known to be effective for 2–3 mo. This bait consisted of packets of ammonium acetate and putrescine, but it contained no edible components.

The design of this test was to compare Mazoferm tent stations with and without the BioLure synthetic baits. The test was performed from December to February. We used toxic bait (containing phloxine B insecticide) and check bait (lacking phloxine B insecticide) in stations with and without the synthetic lure.

The tests were run in the field cage for five consecutive weeks with the same bait stations following the field cage procedure. The four treatments were run in each block of four cages with treatments rotated within the block after each trial. Two bait stations were placed per tree, and 3,000 flies were released into the cage on day 1. Numbers of dead flies were collected from the floor of the cage every 2 h for 4 d. Dead fly collection was made from Monday noon until Friday noon, and then bait stations were removed and surviving flies were trapped until Monday morning. At the end of 5 wk in four of the treatments, the bait was replenished with ≈100 ml of new baits (same formulations) on the sponges, in the other four treatments no new bait was added. Stations receiving fresh bait were compared with those not receiving bait for stations with and without the BioLure packets. Effects of the treatments on numbers of flies killed were analyzed by ANOVA.

Development of Gelled Baits. A gelled bait was developed to address the problems identified with the Mazoferm bait. The goal of developing this bait was to produce a gelled mixture that was effective over a ≥3-mo period. Experiment results described in Moreno and Mangan (1995, 2002) indicated that the Solbait used in sprays was equal or superior to the Mazoferm bait. A new formulation based on a dry hydrolyzed protein, Solulys, (Roquette America, Inc., Keokuk, IA) that is manufactured as a commercial fermentation medium by spray drying Mazoferm. Other additives include dry sugar, Polysorbate 60, ammonium acetate, and various thickeners, conditioners, antifoamants, and humectants (Moreno and Mangan 2002). This material was thickened with vegetable starches and gums (Table 1) to produce a gel. The formulation could be mixed at room temperature and poured into trays for testing. We refer to this mixture as the Solgel bait. This bait was tested for attraction and killing flies over a 10-wk period in the field cage.

Bait stations with the Solgel bait were tested in the eight field-caged trees with each treatment replicated twice in each block of four trees. To measure attraction as well as mortality we attached a strip (4 cm in width) of heavy paper around the bottom circumference of each station and coated the paper on both sides with sticky material. The paper was removed and replaced with freshly coated paper at 2-h intervals from 0900 until 1500 hours daily. At 2-h intervals from ≈0800 until 1600 hours, all dead flies were collected from the bottom of the cage. The two treatments were Solgel baits alone or combined with two (putrescine and ammonium acetate) BioLure packets. Treatments were rotated among trees, but the baits were not changed. Two stations were tested per tree. Data were collected to see whether volatile odors produced were sufficient to attract flies or if addition of BioLure odors would enhance function. Baits were tested for 8 wk, from the last week in February until the second week in April.

Longevity of Solgel Bait. After the demonstration that the Solgel bait alone was sufficient to attract and kill significant portions of the caged fruit fly population, we performed experiments to test the performance of the commercial model (Fig. 1C and D) bait stations over a period of several months. These tests were performed during the winter, spring, and early summer 2001. The trials were carried out by hanging groups of gel bait stations with phloxine B as the toxicant in the ARS orchard from 17 October 2000 to 7 May 2001. Stations were hung on the inside canopy on the east side of grapefruit trees. After baits had aged in the orchard, beginning on 29 January 2001, a set of four of the most aged stations (aged from 17 October 2000), four of the least aged stations (freshly opened), and four of a mid-aged (from 13 December 2000) group of stations were placed in the field cage. Two stations of the same age were hung in each of the field caged grapefruit trees with each block of cages receiving the three age treatments. Two fresh check baits (bait but no toxicant) were hung in the fourth cage in each block. Tests were initiated weekly, stations were hung, and 3,000 reared Mexican fruit flies were released in each of the cages at ≈0700 hours on Mondays. Stations were removed on Fridays at about midmorning and replaced with baited McPhail traps. These traps were removed on Monday at ≈0630 hours before beginning the next weeks testing. The same stations were rotated to new cages each week and the tests were repeated for each group of stations for 14 wk (from 29 January 2001 to 7 May 2001). Total station age was therefore the time that the station was in the field orchard plus the time that the station had been tested in the field cage (ranging from 0 to 187 d of field exposure). Survival was estimated as the ratio of flies recaptured in the treated cage to the average number recaptured in the pair of control cages tested during that trial. This system tests the effects of bait aging in both field and cages and provided estimates of longevity and effectiveness of aged bait for 84 tests.

Results

Bait Stations Compared with Spot Sprays on Trees. Trials of Mazoferm baits (Table 1) in bait stations compared with spot sprays on nursery trees are summarized in Table 2. Mortality occurred on both the trees and the stations, but mortality was relatively low, and recovery of surviving flies in control cages was high. ANOVA of rates of mortality and survival showed highly significant effects of insecticide treatments (both spot sprays and bait stations) compared with control cages measured by counts of dead flies or rates of recovery of surviving flies. Comparisons of check cages for both tree sprays and bait stations showed a highly significant effect of the toxic treatments in reducing flies recaptured ($F = 104.71$; df = 1, 14; $P < 0.001$ for tree applications and $F = 143.3$; df = 1, 14; $P < 0.001$ for station applications) and increasing numbers of dead flies collected from the cage floor ($F = 41.70$; df = 1, 14; $P < 0.001$ for tree applications and $F = 33.26$; df = 1, 14; $P < 0.001$ for station applications). In all tests the tree sprays were more effective (overall 22.5% more effective) than the stations in killing flies when numbers of dead flies collected from

Table 2. Fly response to bait stations and sprayed trees in field cage

Date of test	Control				Toxin			
	Tree		Station		Tree		Station	
	Survive	Dead	Survive	Dead	Survive	Dead	Survive	Dead
18 Mar.	1,137	272	1,508	223	70	1,315.7	171	1,124
22 April	2149	130	2289	102	91.7	1,828	450	994
13 May	1,297	226	1,224	222	67.7	1,449	210	1,262
	Proportion of released flies							
Total	0.509	0.069	0.557	0.054	0.025	0.510	0.097	0.395

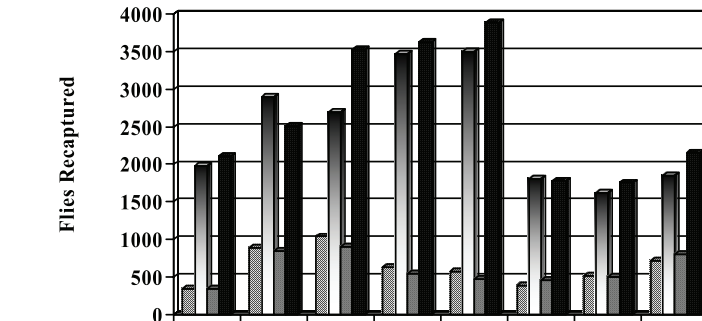
Control treatments were counts from single cages. Toxin data are means of counts from three cages.

the cage floor were compared ($F = 7.02$; df = 1, 16; $P < 0.018$). Numbers of survivors were also significantly different with bait station cages exceeding tree spray cages in the numbers of surviving flies trapped after 4 d of treatment ($F = 14.90$; df = 1, 16; $P < 0.002$) by ≈26%.

Field Trials with Mazoferm Tent Stations. Recapture rates of released sterile flies after 4 d treatment in citrus orchards with Mazoferm bait stations are shown in Fig. 2. Data summarized over all test dates had a means of 2,480.1 recaptured males in the control plots and 632.4 males in the toxic station plots (pairwise $t = 7.39$, df = 7, $P < 0.001$). For females, we recaptured means of 2,670.1 in the control plots and 606.1 recaptures in the bait station plots (pairwise $t = 6.79$, df = 7, $P < 0.001$). Counts of flies recaptured over the eight trials in plots treated with check or toxin bait stations showed that recapture rate was significantly reduced ($F = 47.7$; df = 3, 21; $P < 0.001$) by the toxin. During both summer and fall testing periods, the bait surface on the sponge was moist for the 4-d exposure. We also made observations of activity of insects other than Mexican fruit flies on the baits. Although there was considerable butterfly activity in the orchard, no butterflies were attracted to this bait. Other insects that were relatively common in the orchards included whitefly *Bemisia argentifolii* Perring & Bellows. Although considerable numbers of whiteflies became stuck to the bait surface, this capture was probably due to the large numbers of insects in the orchard rather than attraction to the bait.

Transferred Kill Mortality. Results of tests to determine the effects of bait transfer among flies are given in Fig. 3. Although the differences in survival between the check and insecticide cages for the second populations are not extremely large, differences in survival were repeated across all the replications. The overall reduction of survival in the pesticide cages was ≈18% (mean insecticide pretreatments = 1370.8 recaptures, mean noninsecticide = 1673.5 recaptures), and there was a statistically significant difference between cages with insecticide and those without (pairwise $t = 4.24$, df = 3, $P < 0.025$).

Addition of Synthetic Baits. The effects of supplementing the Mazoferm baits with synthetic attractants are shown in Table 3. The attractant packets (BioLure) containing putrescine and ammonium acetate increased the mortality rates significantly over the test period ($F = 10.84$; df = 1, 4; $P < 0.031$). However, by the fifth week, the mortality rate declined indicating that the bait, the attractant packets, or both compo-



	6-Oct	14-Oct	21-Oct	28-Oct	11-Nov	11-Aug	18-Aug	25-Aug
Toxin Male	345	883	1036	626	571	385	505	708
Control Male	1985	2901	2701	3474	3505	1812	1619	1850
Toxin Female	334	846	903	537	466	459	502	802
Control Female	2113	2516	3531	3630	3887	1783	1756	2151

Fig. 2. Total recaptures for flies released in grapefruit orchard split into bait station treated (≈1-ha) and nontreated (≈1-ha) sections. Approximately 30,000 flies were released per test.

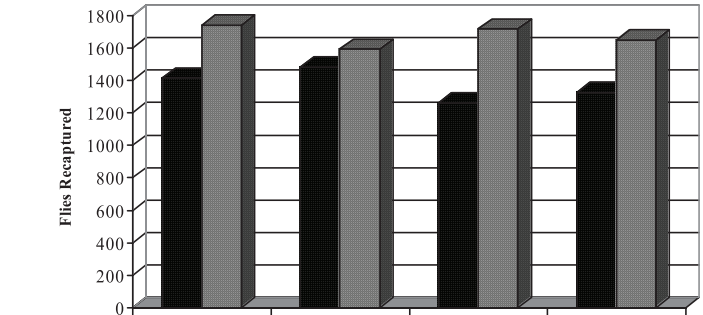


Fig. 3. Survival of flies released into field cages containing flies that had been exposed to toxin or control treated bait stations. Each bar is the sum of four cages.

nents had lost effectiveness. Literature supplied with the bait packets and our experience with the packets indicated that the effective life of these packets was >12 wk. The sponges in the bait stations seemed to be dry and little dye was visible, so we tested whether addition of fresh Mazoferm bait would rejuvenate the stations. Results in Table 3 show that all the stations receiving fresh Mazoferm bait killed far more flies than the stations without the additional bait in the insecticide cages.

Development of Gelled Baits. The persistence of the gelled bait (formula in Table 1) compared with the gelled bait plus the Biolure additives is given in Fig. 4. There was no significant difference in either flies killed (paired $t = 1.02$, df = 8, $P > 0.33$) or fly survival (paired $t = 1.71$, df = 9, $P > 0.21$), and in both tests the numbers killed and numbers surviving showed slightly better results for the bait without the BioLure. These results show that the gelled bait was at least equivalent to the gelled bait plus BioLure attractants for reducing the population.

Longevity of Solgel Bait. Proportions of flies surviving in the treated cages are shown in Fig. 5. Each test (shown as a point on the figure) estimated kill rate for a 4-d exposure period. Estimates of proportion of flies surviving in treated cages showed a weak but significant positive correlation ($r = 0.377$, $P < 0.001$) with the age of the bait. The pattern of survival proportions indicates that kill rate became more variable as the bait aged. The average survival rate for all tests was 27%.

Discussion

A distinct apparatus that kills adult fruit flies by attracting them to a contained poison provides a method for fruit fly population reduction without releasing bait or toxic chemicals into the environment. This method for fruit fly control is an alternative to aerial and ground-applied cover sprays, and to spot sprays. Organic agricultural regions, areas of high public use such as parks, playgrounds and schools, and areas where people do not want to be exposed to sprays or to have their property treated may require this alternative. For a bait station to suppress fruit fly populations in a manner comparable with bait sprays, the material must attract and kill females. The function of the baits depends on production of volatile attractants that are broadcast from a containerized surface that is much smaller than from a sprayed surface. In addition, the attractant must continue to emit volatiles, and the toxicant must retain its activity for a sufficient time to offset the higher cost of station production and placement. The bait must be situated in a station structure that protects the material from weather and intruders, but it will allow attractant volatiles to escape and flies to enter and feed with minimal interference.

Table 3. Numbers of flies killed with Mazoferm bait and BioLure attractants in weekly trials in field cages

Treatment		Nos. killed				
Insecticide bait	Supplementary attractant	Week 1	Week 2	Week 5	Bait refresh	Week 6
Toxin + Mazoferm	BioLure	1,806	1,613	646	Toxin + Mazoferm	1,616
					None	153
Toxin + Mazoferm	None	1,459	1,123	283	Toxin + Mazoferm	1,414
					None	120
Check Mazoferm	BioLure	33	61	45	Check Mazoferm	32
					None	56
Check Mazoferm	None	33	121	40	Check Mazoferm	30
					None	75

Toxic bait in stations was refreshed sixth week in one cage, and stations were maintained with old bait in one cage for each treatment.

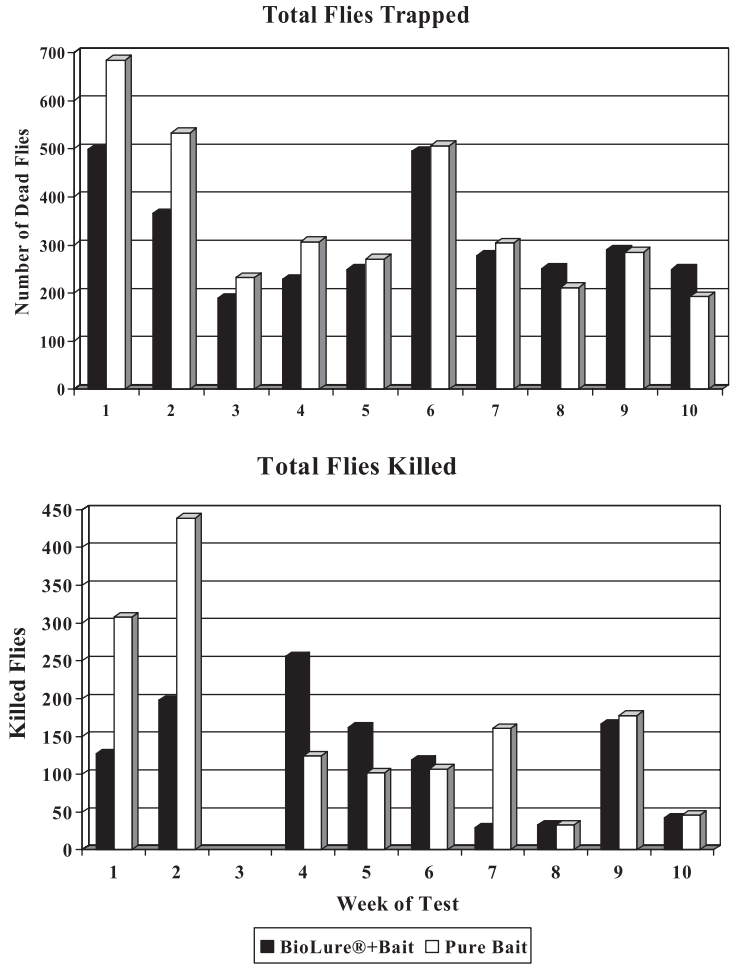


Fig. 4. Total numbers of flies captured and killed over a 10-wk bait trial test using Solbait with or without BioLure supplementary attractants.

Outbreaks of some fruit flies in the genera *Ceratitis* and *Bactrocera* may be controlled by toxic baits composed of parapheromones, which attract and kill males (Cohen and Cohen 1967, Chambers 1977, Cunningham 1989). Drew and Hooper (1981) listed 39 species of *Bactrocera* that respond to methyl eugenol and 88 *Bactrocera* species that respond to cuelure. This method removes the males from the population so reproduction stops and the population is eliminated. The technique is effective for eliminating outbreaks, but it is restricted to conditions with low, spatially defined populations so the entire male population is eliminated. Food-based bait stations can be effective for reducing high populations; these stations that attract and kill both sexes can have impact on fruit fly damage levels, and render the populations vulnerable to other eradication efforts, such as sterile insect release, even when the populations are only partially reduced.

The major disadvantages of using food-based bait stations are the cost of producing and preparing the stations, placement in the field, and the lower effectiveness in comparison with sprayed insecticides that cover larger areas. In this study, we attempted to overcome some of these disadvantages by formulating baits that attract flies over a time exceeding the susceptibility period of most tree-borne fruit. A station and bait also were combined so that additional fly kill could be achieved by action of toxin dispersed by flies away from the station. Cost of the bait and handling difficulties were reduced by using a gel–starch matrix to hold the attractants and toxin rather than bait packets or separate attractant and feeding components.

In 1965–1966, USDA entomologists attempted to reduce *A. ludens* populations by using McPhail traps baited with hydrolyzed torula yeast (Balock and Lopez 1969). Those tests, reviewed in Mangan and Moreno (2002), showed significant reduction in pests trapped in the orchards and fruit infestation levels. The cost of traps and labor and the increase in fruit infestation late in the season suggested that this approach was not feasible. Another series of tests for protein-based attractants was carried out by Ros et al.

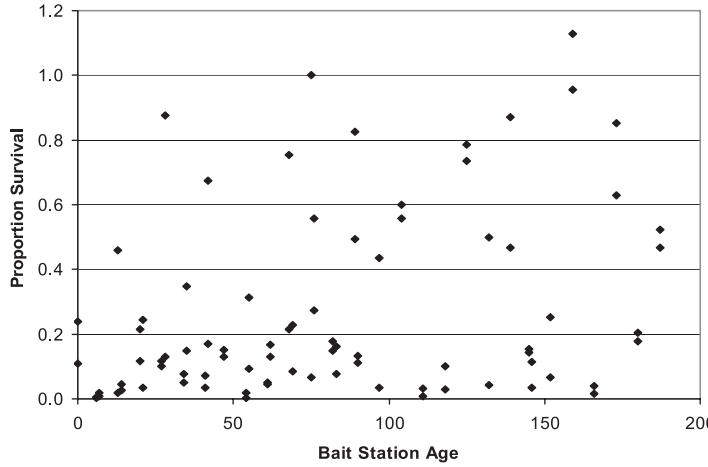


Fig. 5. Proportion of flies recaptured in field cages with baits that were aged various time periods in the Texas grapefruit orchard. Each point is the ratio of number of flies recaptured in cage with bait station to the average number of flies recaptured in two check (nontoxic bait) in adjacent cages.

(2000) against Mediterranean fruit fly. They found that traps with protein-based attractants (BioLure and NuLure) in each tree were effective in reducing fruit damage to cherimoya fruit by as much as 80%.

Sanchez Riviello and Shaw (1966) tested bait stations with protein-based bait (cottonseed protein) to control *A. ludens* in tests carried out in Mexico. That system used the bait to feed flies the chemosterilant tepa (Proverbs 1969) and a green dye marker to produce sterile males that could be identified in the field. They stated that over an eight month period 53% of trapped males in the treated plots had ingested the dyed bait and that reingestion of bait regurgitated from other flies (without the dye) may have increased this number. However, 24 h surveillance of the stations was required due to the "potential danger of tepa bait to man and other animals." They did not mention any concern for human health affected by tepa in the regurgitant accumulating on fruit or contacting humans. In a situation where flies would visit the stations for several months, we would estimate that the effect of flies spreading the insecticide directly to other flies during the lek behavior or by regurgitating the insecticide on plant surfaces could be significant.

Our orchard tests with the liquid Mazoferm-based bait showed that fly populations can be reduced by >80% in a week of exposure with bait stations. In our tests, the density was 50 stations per ha, which is approximately double the density recommended by Arjona (2000) for Mediterranean fruit fly. Although we showed that the Mazoferm bait in protected sponges (tent stations) could effectively reduce the fly populations, further tests indicated that additional attractant chemicals such as those in the BioLure formulations could increase kill rate by the stations. The liquid bait was largely nontoxic by the fifth week, even with the BioLure attractants. The Solgel baits addressed the problems of bait persistence identified with the Mazoferm baits. The use of thickeners, starches, and gels provided a media that could retain moisture and allowed the suspension of additional attractant chemicals. The tests of the gelled baits with and without the BioLure baits showed that the BioLure did not improve attraction to the station.

The tests of longevity of the stations under field conditions showed that for stations < 150 d in the field, the majority of the stations killed >70% of the flies in <5 d. Wild strains of *A. ludens* adults mature in ≈23 d (range 18–33 d) in typical winter conditions in Texas and have minimum maturation time of 8 d under higher temperatures (McPhail and Bliss 1933). From this information, we can extrapolate that over an 8-d maturation period, the expected survival would be <8%. Under the December–April maturation times, the expected survival would range from 0.3% to <0.002%. These survival data are similar to the survival data for the tent stations. The major advantage of the gelled baits is the persistence of ≈150 d compared with ≈21 d for the liquid bait in the tent stations. The longevity of the gelled bait stations in the field demonstrated that the stations could attract and kill flies for the major period of susceptibility to fruit fly attack for most tropical fruit, such as mango, citrus, or guava.

The advances in fruit fly baits, insecticides, and formulations shown by these experiments should enhance the development and use of bait stations. Photoactive dyes or low concentrations of insecticides, such as spinosad, fipronil, or imidacloprid (Moreno and Mangan 2002), reduce the vertebrate toxicity of the baits to levels thousands of times lower than organophosphate baits. The noninsecticide components in the gelled bait tested here are approved for human consumption. The bait stations composed of liquid organophosphate insecticide and hydrolyzed protein baits have a projected field effectiveness of one week (Arjona 2000). Addition of oils, humectants, and conditioners improve the effective period to ≈3 wk for the Mazoferm bait, and addition of gelling agents, thickeners, and further refinements extend this period to 16 wk for the Solgel bait. The Solgel components also

allow mixing without heating. Baits can be prepared and poured into bait trays by mixing the dry components with water thus reducing shipping and storage costs compared with the premixed liquid baits.

We have not addressed several key questions for use of these types of stations. Probably the most important factor is station density. Station density will require extensive testing and will probably be determined by habitat conditions and fruit fly species. The final tests concerning effects of aging used one station per tree in an enclosed cage. Our formulations were designed to maximize both attraction of the bait and feeding with the goal of controlling populations by maximizing attraction. Preliminary field tests in Nuevo Leon, Mexico, have shown that with orchard densities of 16–32 stations per ha, we can attract and kill significant numbers of flies. However, fruit infestation and numbers of wild flies captured in surveillance traps did not decline and in several cases increased. This pattern suggested that we were attracting flies into the orchard but not killing them. An areawide bait station program may be necessary to reduce complete populations in the surrounding community to reduce entire populations.

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